Effects of Salinity on Survival, Growth and Reproduction of the Water Flea, *Daphnia magna*

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ABSTRACT: Recent advancing industrialization and urbanization have increased salt concentrations in formerly-freshwater habitats. Freshwater animals are being affected, especially those like crustaceans that are unable to emigrate to escape the problem. *Daphnia magna* is mainly recognized as a freshwater cladoceran, but there are some strains that grow in brackish waters. There has been an increasing interest in using the freshwater crustacean *Daphnia magna* as a toxicological test species for water systems. The determined 48h-LC50s from studied salinities were 2.99, 3.92 and 4.82 ‰, for Sodium chloride (NaCl), synthetic sea water and filtered natural sea water respectively. The effect of salinity on reproduction, growth and survival rates of *D. magna* was studied under laboratory conditions. All individuals were fed with *Scenedesmus obliquus*. Salinity effects on daphnids was investigated at sublethal salinities LC10, LC15, LC20, LC25, LC30, LC35, and LC40 for the three tested saline waters. The number of progeny per female was the most at 0.44‰ (LC10) for synthetic sea water, at 1.67‰ (LC10) for NaCl, but for natural sea water at 2.48‰ (LC10) and 2.82‰ (LC15) were more or less like that of control. Time to the first brood was 9, 9 and 8 for the tested waters respectively indicating delay because of salinities effects when compared with those of control which was 7 days. Survival and growth rates of daphnids were decreased with increasing salinities of the three tested waters. Among three salinity series tested, daphnid survival rate was the highest in control; 97% followed by 80-90 % at LC10 after 21 days experimental period. The highest total reproduction 95±9 neonates per female (mean ±S.D.) were achieved at 0.44 ‰ (LC10) for synthetic sea water, over a period of 21 days. Data showed that, groups of *D. magna* reared in synthetic sea water 0.44‰ showed highest length and specific growth rate. Four essential amino acids were detected for *D. magna* reared in 0.44‰ synthetic sea water while for control group only two. Fatty acids profile of *D. magna* showed four groups of saturated fatty acids dominated by myristic acid (14:0). Moreover, unsaturated fatty acids (USFAs) represented by nine groups and the most abundant USFAs was linoleic acid (18:2n-6).From this study, it is concluded that the strain of *Daphnia magna* used in our study can not withstand high salinities; it may need a long period of time to accommodate to such high salinities to be maintained in aquacultures to be used as live food for higher crustaceans or various species of fish. Besides, low salinities at LC10 (0.44‰) for synthetic sea water, or below may enhance reproduction for this test organism. [Nature and Science. 2009;7(11):28-41]. (ISSN: 1545-0740).

Keywords: Salinity, Sodium Chloride, Synthetic and Natural Sea waters, *Daphnia magna*, Survival, Growth, Reproduction

INTRODUCTION

Since saline and freshwater environments require completely different adaptations if the animals inhabiting them are to retain suitable osmotic pressure and cell homeostasis, most aquatic organisms are unambiguously characteristic of either one habitat or the other (Young *et al.* 1989). Nevertheless, as salinity varies markedly in many habitats, such as estuaries or coastal lakes (Hall and Burns 2002, Schallenberg *et al.* 2003), local populations may be characterized by the presence of micro-evolutionary changes, the widening of tolerance ranges and greater phenotypic plasticity.

Freshwater invertebrates have been submitted over a long period of time to selection to cope successfully with the low osmotic pressure of their present habitat. Their adaptations to live in low salt concentrations and low osmotic pressure, however, are presently being subject to an unexpected test in waters enriched with salts due to ongoing industrialization and urbanization, e.g. with mine waters and storm drain runoff from streets treated with salt in winter. As the tolerance (or lack of tolerance) to high salinity in freshwater animals is poorly recognized, particularly in invertebrates which are not mobile enough to swim away from affected habitats.

Zooplankton production plays an important role in the functioning of aquatic ecosystems by making part of the production of phytoplankton available to higher trophic levels. Crustaceans, which often dominate the zooplankton are the major herbivores in

many aquatic communities and are the main food for bigger crustaceans, various species of fish and representative from many other taxa.

Cladocerans are very important components of zooplankton, usually restricted to freshwater environments (Arnér and Koivisto, 1993) with salinity values lower than 1gL^{-1} (Hart *et al*., 1991) or conductivity values less than 500 mS cm^{-1} (Hebert *et al.*, 2002). The genus *Daphnia* is freshwater in its origin and distribution (Peters, 1987; Teschner, 1995); for North America, there are 34 species in freshwater environments and only one for saline lakes; *Daphnia salina* (Hebert *et al*., 2002). *Daphnia* are hypertonic to the medium and the fluxes of water and solutes with the surrounding water could be considerable, but they have reduced their osmotic loads through the impermeability of their bodies and the low internal concentration of solutes, being sodium pumping from the epithelial cytoplasm to the hemolymph the major mechanism for osmoregulation in freshwater cladocerans (Peters, 1987). According to Arnér and Koivisto (1993), although it is possible to find *D. magna* in rock pools with salinity values up to 12.5% in the Baltic Sea, they experimentally determined that the best development was achieved at 4%. Schuytema *et al.* (1997) also concluded that the best growth of *D. magna* occurs at salinity values lower than 4‰. The freshwater Cladocera that successfully colonize brackish environments are smaller in size and have a reduced reproduction (Arnér and Koivisto, 1993). Cowgill and Milazzo (1990, 1991) demonstrated that reproduction, population growth rate, and survival in *D. magna* decreased as NaCl concentration increased in the range of 0.08–6000 mgL^{-1}.

There is relatively little information available on the responses and adaptations of freshwater organisms penetrating into brackish water. Among cladocerans, a great majority are exclusively freshwater animal, although a few genera such as *Podon, Evodne, Bosmina* and *Penilia* , have recently colonized brackish and marine environments and some species like *Moina hutchinsoni* and *Daphniopsis pusilla* live in saline lakes (Potts and Durning, 1980). In previous studies *Daphnia magna* has been found in springs of high salinities up to 62‰ (Ghazy, 2003).

Martínez-Jerónimo and Martínez-Jerónimo (2007) also stated that *Daphnia magna* is mainly recognized as a freshwater cladoceran, but there are some strains that grow in brackish waters. Some species have been observed in salinities up to 4 ppt, and salinities of 1.5 to 3ppt are common in pond cultures in the orient.

Even though salinity is one of the niche dimensions that affect the distribution of *D. magna*, there are only a few experimental studies on the salinity tolerance of *D. magna* (Lagerspetz, 1955; Cowgill and Milazzo 1990, 1991).

The aim of this study was to investigate the effect of salinity of each of sodium chloride as a model compound, synthetic sea water and natural sea water, on survival, growth and reproduction of *Daphnia magna* and to establish the maximum salinity level in which *Daphnia* can survive and reproduce to be used as a natural food in aquaculture where *Daphnia magna* is of high nutritive value for aquatic animals.

MATERIALS AND METHODS

Experimental animals and food:

A freshwater *D. magna* strain that has been successfully grown in our laboratory of Hydrobiology in National Research Center for more than 19 years in synthetic freshwater media (Fayed and Ghazy, 2000) was used as the test organism for this study.

Gravid females were transferred at regular intervals to 1-L glass beakers, in which the culture medium; synthetic freshwater medium (pH; 7.9, total hardness; 90 mg/L as Ca CO_3, alkalinity; 34 mg/L as Ca CO_3, conductivity; 260µmhos) was renewed 3 times a week and were checked daily for the release of neonates to be used in starting experiments. In these beakers, the animals were fed 3 times a week with 14X10^7 cells/ml of the green micro alga *Scenedesmus obliquus*, it was previously determined that this cell concentrations is an optimal food dosage for this strain (Ghazy,1997). The algal culture was renewed once a week to maintain the algae solution in good condition. The algae and the daphnids were kept at a temperature 22± 2°C with a light period of 16 L: 8 D both during culturing and experimental periods.

Facilities and protocols:

The experiments were carried out in 250-ml glass beakers contained 100 ml synthetic freshwater media for control and inoculated with 10 neonates < 24 h.

The effect of salinity treatments were conducted using three test waters; sodium chloride (NaCl) solution, Synthetic sea water (Instant Ocean® Salt, Aquarium Systems,France) and natural filtered sea water. Every treatment ran in parallel with control in three replicates, each replicate contained 10 neonates in 100 ml test water in 250 ml glass beakers. The natural or artificial test water was diluted with the synthetic freshwater media to the respective test salinity. Test media were prepared by diluting saline water with synthetic freshwater media until the

required salinities were recorded with a salinity-conductivity-temperature Meter (YSI Model 33).

Temperature in a 70x60x30 cm aquarium in which test beakers were conducted, was maintained constant by automatic heater (thermostat), Model "hydor",Italy. A mercury thermometer was used to measure temperature in test containers to be at 22±2° C. Natural day length during the experiment period was 16L: 8D. Synthetic freshwater media was used as dilution water and for control.

Methods

Acute tests:

Acute toxicity testing were in triplicates where groups of 10 < 24 h-old daphnids are placed in 250-ml beakers, each containing 100 ml medium and subjected to test conditions for 48 h. Tests were run without food addition. The number of live organisms after the elapse of 48h is recorded. Control test is run in parallel. Salinities series 0, 2, 3, 4, and 5‰ (parts per thousand, ppt) for NaCl solution, 2, 4, 6, 8, 10, 20 ‰ for synthetic sea water and 3.5, 4, 4,2, 5.8, 6.1 ‰ for filtered natural sea water, were studied.

Chronic tests:

Ten neonates (<24h-old, standard length of 1.60 to 2.00 mm) were placed in each 250 ml - glass beakers containing 100 ml of synthetic freshwater for control or saline water for each treatment which were renewed with addition of fresh food three times a week .These experiments lasted for 21 days. Tests were run with food addition three times a week during changing test water.

Salinities effects on daphnids was investigated at series: 1.67, 1.86, 2.04, 2.20, 2.35, 2.51, 2.66 ‰ for NaCl, for synthetic sea water 0.44, 0.67, 0.93, 1.24, 1.60, 2.03, 2.54 ‰, and for natural filtered sea water at 2.48, 2.82, 3.12, 3.40, 3.68, 3.95, 4.42 ‰ which were corresponding to sub-lethal salinities LC10, LC15, LC20, LC25, LC30, LC35, LC40 for each series, determined from acute tests.

For chronic tests, three times a week, *Daphnia* were removed from their container and placed immediately into a new prepared synthetic freshwater media, as control and different salinity-adjusted treatments containing algal food, Scenedesmus *obliquus* at $14x10^7$ coenobia/ ml.

Survival, growth and reproduction rates of daphnids were recorded three times a week. The survival rate was calculated by dividing the numbers counted every time by the number of neonates at the beginning of the experiment.

Growth was determined from the body lengths which were measured under the microscope with an ocular micrometer (160 X magnification) from base of caudal spine to the anterior edge of the head.

Growth is described as the increase in body length over time. Growth in crustaceans is a discontinuous process, i.e. the succession of molts (= exuvia, ecdyses) is separated by intermoult periods. Each time an individual moults , the old integument is shed and a rapid, extensive growth occurs during the short period before the standard length at subsequent moults was tested in function of salinity using a repeated measures analysis of variance (ANOVA).

The age at release of first brood was noted. After every reproduction the offspring were counted and taken away until end of experiment to calculate the number of progeny per *D. magna* female.

Biochemical analysis:

At the end of experiment the biochemical composition (proximate analysis), amino and fatty acids of *D. magna* were determined for the best concentration of synthetic sea water LC10 (0.44‰) and control (0‰).

Proximate analysis: Protein content of daphnids was determined according to Daughaday *et al*. (1952). Lipid content was determined according to the method of Knight *et al*. (1972). Ash and moisture were analyzed according to the method of AOAC (1999).

Analysis of amino acids: The sample was ground and filtered. The residue was washed with a few ml of 75% ethanol and the volume was made up to 100 ml. Several amino acids were examined using a HPLC system (HP1050) with a UV detector at 254 nm. The separation was accomplished with an APS, NH2, (5 µm. 4 ×250 mm) column. The mobile phase consists of 32% (methanol/water), 60/40 with 0.3 ml acetic acid. The flow rate was 0.9 ml/min. The temperature of column was 45°C, while the injection volume was one µl according to the method of (Christian, 1990).

Analysis of Fatty acids: Lipids were extracted from daphnids using the procedure of Folch *et al*. (1957) by homogenizing them in a mechanical blender with a mixture of chloroform and methanol (2:1 v/v). To prevent oxidation, crystals of hydroquinone were added to all samples. The chloroform extract was evaporated at 55ºC under vacuum and the residue weighed.

Following the extraction of lipids from daphnids, methyl esters of fatty acids were prepared for subsequent use in gas-liquid chromatography. Lipid extracts were converted to their methyl esters according to Hartman and Lago (1973).

Analysis of methyl esters were performed on a

CG-17 Gas Chromatography (CG Instrumentos, Sao Paulo, Brazil), equipped with a flame ionization detector.

A stainless steel column, 2m x 5mm, packed with chromosorb W coated with 18% (by wt) of diethylene glycol succinate (DEGS) was used. The operating conditions were as follows: column temperature, 195ºC; sample vaporizer temperature, 225ºC; detector temperature, 245ºC. The carrier gas used was nitrogen, at a flow rate of 40 ml/min. Injected sample size were in the range 2.0-3.0 ml. Fatty acids were identified by comparison with the retention time of standards and by equivalent chain length (Ackman, 1969).

Statistical Analyses:

Probit Analysis was used to calculate the 48h-LC50s for acute tests and 21day - LC50s for chronic tests on *Daphnia magna*, from studied salinities as described by Finney's method (1977). The terminology recommended by Sprague (1969), lethal concentration (LC) was used for survival and, as given here, represents an interpolation from three or more partial-effect concentrations.

Data were analyzed by ANOVA using the SAS ANOVA procedure (SAS, 1988). Fisher′s least significant difference test was used to compare treatment means.

RESULTS AND DISCUSSION

Median lethal concentrations:

The probit estimated 48h-LC50 for NaCl was 2.99 ‰, the tested concentrations ranged from 0 to 6 ‰, for Synthetic sea water and natural sea water 48h-LC50s were 3.92 and 4.82 ‰, and their tested concentrations ranged from 0 to 22‰ and 0 to 6.2, respectively (Table 1) and at the highest salinity, in all the tests, all neonates died during 48 hours. Mortality rates are also elevated where the salt concentration is high, though susceptibility to salt differs both between species and between clones of the same species (Grzesiuk& Mikulski, 2006). The probit estimated 21d-LC50s for the three tested waters were 2.54, 2.27 and 3.48 ‰ respectively (Table 1).

Table (1): Comparison between effects of salinities of each of sodium chloride (NaCl), synthetic sea water and natural filtered sea water in acute (48h) and chronic (21 days) tests

Toxicity (LC)	Salinity (‰)					
	NaCl		Synthetic Sea Water		Natural Sea Water	
	48h-acute test	21day-chronic test	48h-acute test	21day-chronic test	48h-acute test	21day-chronic test
LC10	1.66	1.70	0.44	0.43	2.48	2.14
LC15	1.86	1.83	0.67	0.58	2.82	2.34
LC20	2.04	1.95	0.93	0.76	3.12	2.52
LC25	2.20	2.05	1.24	0.94	3.40	2.69
LC30	2.35	2.15	1.60	1.15	3.68	2.85
LC35	2.51	2.25	2.03	1.37	3.95	3.00
LC40	2.66	2.34	2.54	1.63	4.23	3.16
LC45	2.82	2.44	3.16	1.93	4.52	3.31
LC50	2.99	2.54	3.92	2.27	4.82	3.48

The effect of salinities on the survival, growth and reproduction rate of *D. magna*:

Effect of NaCl salinity:

Table (2) and Fig. (1) shows the effect of different sub-lethal concentrations of NaCl on survival rate of *D. magna* at the end of experimental, generally it was found that the survival rate decreased with increasing the cultured period and also with increasing the concentrations from 0‰ (control) to 2.66 ‰ (LC40). Groups of *D. magna* cultured in 0‰ showed the highest significant ($P < 0.01$) survival rate represented by 97% after 21 days. While the lowest significant survival rate ($P < 0.0001$) for those cultured in the highest concentration, 2.66‰ represented by 43% after 21 days. There were no significant differences between control and LC10 groups. Strong negative correlations was observed from the first 2 days of the experiment $r = -0.69$ and increased till the end of experiment to reach $r = -0.99$ at $P<0.005$.

Table (2): Effect of salinity as sodium chloride, NaCl on % survival, growth and reproduction rate of *Daphnia magna* cultured in static renewal system for 21 days.

NaCl addition to exposure medium (‰)	**% survival at 21st day**	**Time to the first brood (days)**	**Number of progeny per female at 21st day (mean±SD)**	**Mean length of adult females at 21st day in mm (mean±SD)**	**Average weight of adult females at 21st day (mg)**	
					Wet weight	**Dry weight**
Control	97[a]	7	(40±24)[a]	(3.74±0.06)[a]	8.7	0.322
1.67LC10	90[ab]	9	(54±9)[b]	(3.73±0.08)[a]	8.3	0.375
1.86LC15	83[bc]	9	(49±6)[a]	(3.70±0.00)[a]	7.6	0.369
2.04LC20	77[c]	9	(49±7)[a]	(3.61±0.07)[b]	7.5	0.325
2.20LC25	70[c]	9	(48±8)[a]	(3.59±0.29)[b]	7.5	0.288
2.35LC30	60[d]	9	(45±9)[a]	(3.55±0.20)[b]	7.2	0.272
2.51LC35	50[ef]	9	(33±7)[c]	(3.42±0.13)[c]	6.4	0.230
2.66LC40	43[f]	9	(32±3)[c]	(3.38±0.07)[c]	6.2	0.220

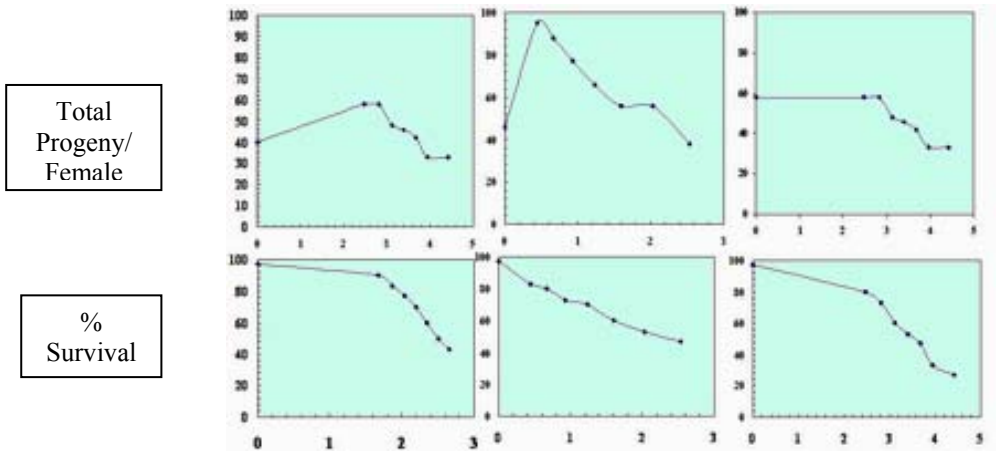
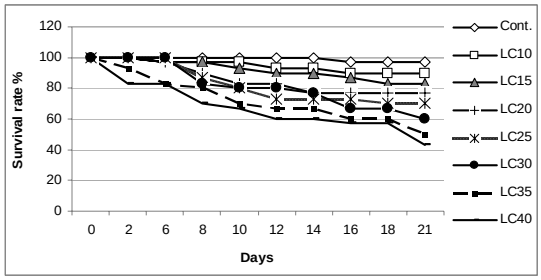
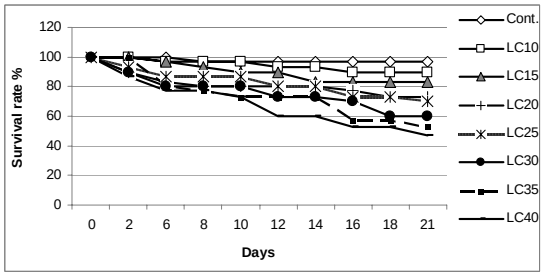


Fig. (1) Survival and Reproduction of *Daphnia magna* grown at different salinities at the 21st day

Table (2) and Figure (3) illustrate the effect of different concentrations of NaCl on growth rate of *D. magna* at the end of experiment. Generally it was found that the length of *D. magna* increased with increasing period of culture for all concentrations. At the end of experiment, control group (0‰ salinity) showed the highest significant (P<0.001) lengths which represented by 3.74mm. While the lowest significant (P<0.001) lengths were observed for group cultured at the highest NaCl concentrations (2.66 ‰) corresponding to LC 40, which represented by 3.38 mm after 21 days. There were no significant differences (P<0.001) between control group and `groups cultured in concentrations 1.67, 1.86 and 2.04‰ corresponding to LC10, LC15, LC20, respectively.



Synthetic Sea Water



Nature Sea Water

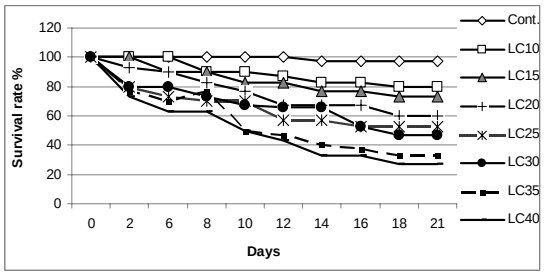


Figure (2): Effect of different concentrations of NaCl, synthetic sea water, and nature sea water on survival rate (mean±SE) of *D. magna* during the experimental periods (21 days).

Salinity affected reproduction of females during the experiments the number of progeny per female at 21 day for NaCl salinity (Table 2 and Figure 1) was highest (54 neonates/female) at 1.67‰ corresponding to LC10, but not differ significantly (P>0.01) than those reared on 1.86‰, 2.04‰, 2.20‰ and 2.35‰. The lowest number of progeny (32 neonates/female) was observed for those reared at the highest concentration (2.66‰) corresponding to LC40. So it was noticed that the number of progeny per female decreased as salinity increased. Arnér and Koivisto (1993) demonstrated that the freshwater Cladocera that successfully colonize brackish environments are smaller in size and have a reduced reproduction. Cowgill and Milazzo (1990, 1991) demonstrated that reproduction, population growth rate, and survival in *D. magna* decreased as NaCl concentration increased in the range of 0.08–6000 mgL^{-1}.

Effect of synthetic sea water salinity:

Effect of different salinity treatments of synthetic sea water on survival rate of *D. magna* during the experimental period is in Fig. (2). In general the survival rate decreased with increasing the culture periods and increasing the concentrations of salinity, the highest survival rate (97%) noticed for control groups, followed by those cultured in LC10. Also, survival decreased gradually as the concentration of salinity increased until the highest concentration (2.54‰) corresponding to LC40, where the survival rate was 47%. From the first two days data showed negative correlation r = -0.57 and increased as the experiment progress r = -0.93 at P<0.005 (Table 3 and Fig. 2).

From Figure (3), it is clear that the length of *D. magna* increased with increasing culture period for all concentrations. It was found that the highest length; 3.88 mm was observed for group reared in 0.44‰ (LC10) which differed significantly (p<0.001) than those reared in the other concentrations. While the lowest length was found for those reared at the highest concentration; 2.54‰ corresponding to LC40 represented by 3.28 mm.

Mean total progeny per female, over the experiment period ranged from 38 to 95 neonates/female .The maximum significant count (P<0.01) was at LC10, and represented by 95 neonates/female, which differ than the other concentrations even the control group. The age at first reproduction was 9 days for all treatments but was 7 days for control (Table 3 & Fig. 1).

Table (3): Effect of synthetic sea water salinity on % survival, growth and reproduction rate of *Daphnia magna* cultured in static- renewal system for 21 days.

Synthetic sea water addition to exposure medium (‰)	% survival at 21st day	Time to the first brood (days)	Number of progeny per female at 21st day mean±SD)	Mean length of adult females at 21st day in mm (mean±SD)	Average weight of adult females at 21st day (mg) Wet weight	Dry weight
Control	97[a]	7	(46±7)[a]	(3.35±0.27)[a]	7.8	0.288
0.44LC10	83[bc]	9	(95±9)[b]	(3.88±0.13)[b]	7.9	0.292
0.67LC15	80[c]	9	(88±7)[c]	(3.74±0.13)[c]	7.8	0.288
0.93LC20	73[c]	9	(77±7)[d]	(3.42±0.21)[a]	7.4	0.273
1.24LC25	70[c]	9	(66±8)[e]	(3.40±0.3)[a]	7.0	0.265
1.60LC30	60[d]	9	(56±9)[f]	(3.40±0.10)[a]	6.9	0.259
2.03LC35	53[de]	9	(56±7)[f]	(3.35±0.13)[a]	6.3	0.255
2.54LC40	47[e]	9	(38±6)[g]	(3.28±0.2)[a]	5.5	0.225

Such delays in the onset of reproduction have been observed for salt-stressed horseshoe crabs *Limulus polyphemus* (L.) (Ehlinger and Tankersley 2004), copepods of the species *Gladioferens imparipes* Thompson (Payne and Rippingale 2001) and cladocerans *Daphnia carinata* King (Hall and Burns 2002) and *D. magna* (Arner and Koivisto 1993). Salinity can cause both delayed maturity and a smaller size at first reproduction, as Teschner (1995) showed for *Daphnia magna*.

Effect of natural sea water salinity on *D. magna*:

For natural sea water treatments, the salinity could affect the survival rate of *D. magna* (Fig. 1). At the end of the experiment, the highest significant survival rate (P<0.0001) for control group (0‰), followed by those cultured in the lowest salinity, 2.48‰ (LC10) were represented by 97 and 80%, respectively. While the lowest significant survival rate (27‰) at (P<0.0001) was observed for those cultured in the highest salinity of 4.42‰ corresponding to LC40 (Table 4).

Figure (3) indicate the effect of different salinities of natural sea water on the growth rate of *D. magna* during the experimental period. It was found that the length of *D. magna* increased for all different concentrations with increasing culture period.

For control group (0‰ salinity) the daphnids lengths was 3.76 mm at the end of experiment which differed significantly (P<0.01) than the groups cultured in different concentrations except for group cultured in concentration 2.48 ‰ corresponding to LC10, it was found that length of *D. magna* not significantly differed (P>0.01) between control group and those cultured in 2.48‰ (LC10). While the lowest significant length (p<0.01) was observed for those cultured in the highest concentration represented by 3.23 mm (Table 4). The highest production of offspring per female at the end of experiment (58 neonates/female) were observed at 0, 2.48 and 2.82‰, which did not differ significantly (P>0.01). However, the production declined gradually with increasing salinity till reach the lowest significant value (P<0.01); 33 neonates/female at 4.42‰ (Table 4 and Fig. 1).

Thus the growth, survival and reproduction rate of the adult daphnids decreased in a high salinity environment. It is known that *D. magna* is a freshwater crustacean organism and it is adapted to low salinity condition, but on the other hand, it was found in a previous study (Ghazy, 2003) that a strain of *D. magna* could survive in salinities up to 62‰ in El-Imam El-Shaffie spring.

Table (4): Effect of natural sea water salinity on % survival, growth and reproduction rate of *Daphnia magna* cultured in static renewal system for 21 days.

Natural sea water addition to exposure medium (‰)	% survival at 21st day	Time to the first brood (days)	Number of progeny per female at 21st day (mean±SD)	Mean length of adult females at 21st day in mm (mean±SD)	Average weight of adult females at 21st day (mg)	
					Wet weight	Dry weight
Control	97[a]	7	(58±2.8)[a]	(3.76±0.18)[a]	8.7	0.32
2.48LC10	80[b]	8	(58±3.0)[a]	(3.70±0.23)[ab]	8.1	0.30
2.82LC15	73[b]	8	(58±1.00)[a]	(3.69±0.25)[b]	7.5	0.28
3.12LC20	60[c]	8	(48±2.0)[b]	(3.52±0.22)[c]	7.2	0.28
3.40LC25	53[c]	8	(46±1.8)[b]	(3.48±0.25)[c]	7.0	0.26
3.68LC30	47[e]	8	(42±2.7)[b]	(3.36±0.05)[d]	6.9	0.25
3.95LC35	33[f]	8	(33±2.1)[c]	(3.35±0.11)[d]	6.6	0.23
4.42LC40	27[f]	8	(33±2.0)[c]	(3.23±0.07)[e]	6.1	0.23

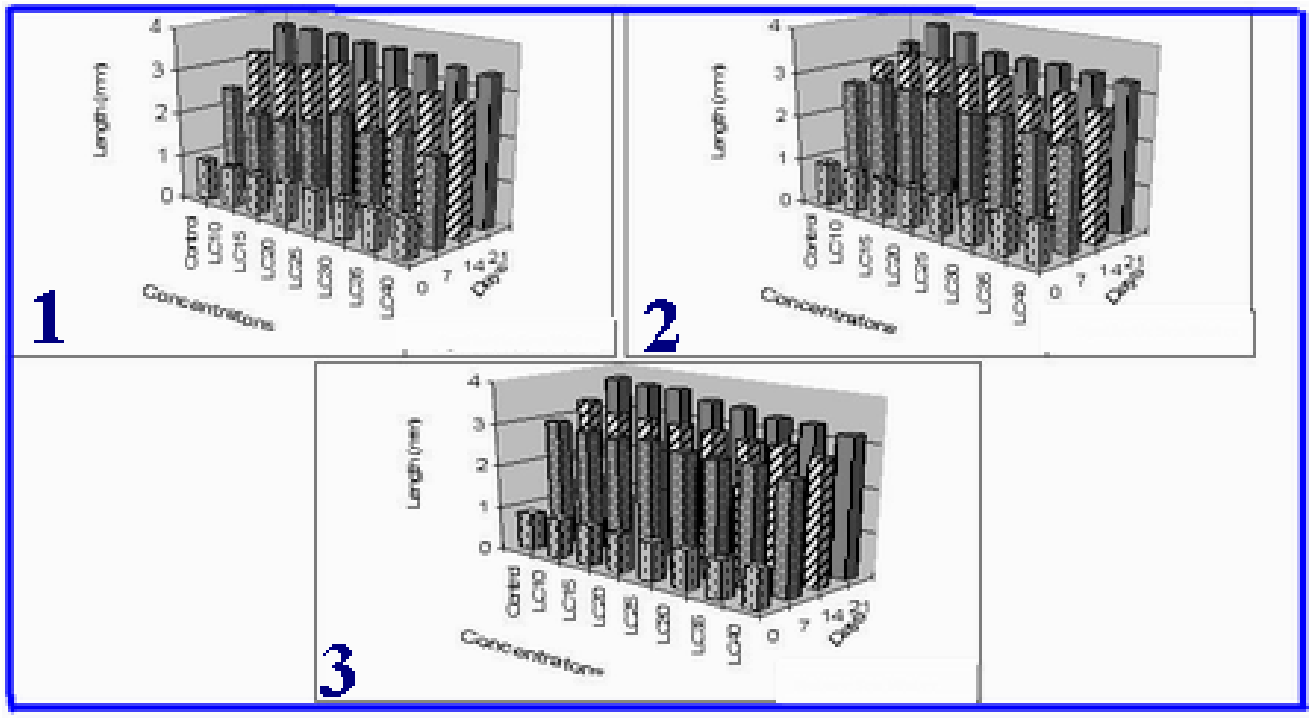


Figure (3): Effect of different concentrations of NaCl, synthetic sea water, and nature sea water on survival rate of *D.magna* during the experimental period (21 days), 1= Sodium Chloride, 2= Synthetic Sea Water, 3= Nature Sea Water

Comparing results in the three concentration ranges tested, we can conclude that the impairment effects on reproduction and survival under the current treatments were provoked by salinity stress. It has been demonstrated that various freshwater invertebrate species (including cladocerans) are more sensitive to NaCl salinity than to the effect produced by the array of chemical compounds present in sea salt (Kefford *et al*., 2004). In this study, *D. magna* is more sensitive to NaCl salinities than to that of synthetic- and natural sea waters.

The present results might be useful for a better understanding of how salinity affected the survival, growth and reproductive responses of a freshwater *D. magna* strain. These data provide support for the use of this strain, based on this capability to endure brackish waters, as test organism in toxicity assays performed in slightly saline conditions (up to 2.99, 3.92 or 4.82‰ for the three salinities studied).

Casey *et al.* (2000) stated that the LC50 lethal salt concentration for *Daphnia magna* varies from 5491 to 5736 mg NaCl L-1).

Also, for *D. magna*, Schuytema *et al*. (1997) determined a median lethal concentration (LC50) at salinity concentration of 6.6 gL_1 (measured conductivity, 10.0mScm_1). Cowgill and Milazzo (1990) estimated an LC50 for NaCl at 6.034 gL_1, and pointed out that the ‘‘non observable effects level’’ (NOEL) for NaCl was approximately 1.2 gL_1. In the present study, 48h-LC50 was determined to occur at the NaCl concentration of 2.99‰, and was lower than previously reported values. This situation could be related to a greater sensitivity to salinity by the experimental strain we used, a plausible situation since this strain has been grown with outstanding reproductive results in freshwater conditions for more than 15 years. On the other hand, Arnér and Koivisto (1993) reported that *D. magna* grew in salinities of 4‰ and 8‰.

Kikuchi (1983) stated that the gills and digestive tracts of crustaceans are their basic osmoregulatory organs, with changes in salinity capable of modifying gill morphology in *Daphnia*, for example. These changes affect the so-called dark cells in particular, these being rich in mitochondria and possessed of an elaborate tubular system and modified cell membrane. It is probable that they play an important role in osmoregulation.

Aladin (1991) described round nuchal (neck) organs in *Daphnia magna* embryos, whose cytoplasm is seen to be very condensed and capable of intensive cellular absorption of salt on account of high permeability to ions. Adult *Daphnia* do not rely on such neck organs, relying instead on the absorption of salt with food.

Williams (1998) remarked that *D. magna* in slightly brackish waters has a narrow range of salt tolerance, whereas varieties found in highly saline lakes display a wide tolerance to this factor, as observed in *Moina hutchinsoni*, a cladoceran that flourishes in saline lakes in North America at up to 40 psu, but *M. hutchinsoni* can be grown under laboratory conditions at salinity values as low as 4 psu with similar results to those recorded at a higher salinity (Martínez-Jerónimo *et al*., 2004; Martínez- Jerónimo and Espinosa-Chávez, 2005).

Considering its tolerance to salinity, Green *et al*. (2005) established that *D. magna* is a euryhaline species that is particularly tolerant to salinity conditions in brackish lakes; nevertheless, they concluded that the reproductive and/or survival rates of cladocerans are reduced at higher water conductivities. In our study, we demonstrated that the freshwater strain we used has in fact a relatively small range of tolerance to studied salinities; nevertheless there was a strain of *D. magna* has been acclimated to thrive at the upper salinity values (Ghazy,2003).

It was found that two published examples of behavioral responses to salinity in freshwater crustaceans were studied. First, Baillieut and Blust (1999) found that high salinity level reduced swimming speed in *Daphnia magna*. Harder (1968) observed aggregation behavior in zooplankton responding to increased salinity but did not suggest any advantages in terms of fitness.

Grzesiuk and Mikulski (2006) stated that the effect of salinity can be modified by other abiotic factors, albeit with the pattern of these modifications varying. A strong interaction between effects of temperature and salinity on survival of *Daphnia magna* has been demonstrated, a high temperature compounding the harmful effect of the salinity (Casey *et al.* 2000). Even where it does not reduce lifespan, salinity may limit individuals’ growth rates, with freshwater animals transferred to a brackish environment found to grow more slowly: as with *Daphnia carinata* (Hall and Burns 2002) and *D. magna* (Teschner 1995, Arner and Koivisto 1993).

Biochemical composition of *Daphnia magna*

The protein, lipid, ash and Moisture content of *D. magna* reared in 0‰ (control group) and 0.44‰ corresponding to LC10 synthetic sea water are given in Table (5).

Table (5): Biochemical composition of *Daphnia magna* reared in 0‰ (control) and 0.44‰ (LC10) synthetic sea water (g/100g wet weight)

Treatment	Control (0‰)	Synthetic sea water LC10(0.44‰)
Total protein	4.18±0.63	5.2±0.85
Total lipid	1.09±0.135	1.15±0.02
Moisture%	81±2.5	79±3.1
Ash%	8.7±1.22	8.8±1.5

Values are given as means ± SE for triplicate determination

Both groups of *D. magna* had high moisture (81% and 79%, respectively). Protein, lipid and Ash content slightly increased for *D. magna* reared in 0.44‰ than those reared in control group and are represented by 5.2(g/100g wet weight), 1.15(g/100g wet weight) and 8.8%, respectively. A similar results was obtained by Habashy (1998) suggesting that the composition of the same species (*D. magna*) was 6.9 (g/100g wet weight) for protein content, 0.91 (g/100g wet weight) for the total lipid, 8.9% Ash and 84.6% for moisture. In this respect, other studies recorded the protein content of *Daphnia carinata* and *Moina australiensis* is 54.34% and 64.80%, respectively (Kibria *et al*., 1999) and in *Daphnia* sp. it is reported to be 49.70% (Yurkowski and Tabachek, 1979; Watanabe *et al*., 1983), whereas for *Moina* it varies between 59% and 77.85% (Tay *et al*., 1991). The present investigation showed that protein content of both groups of *D. magna* were 4.18 and 5.2 g/100g wet weight which is lower than reported earlier (Tay *et al*., 1991) which may be due to analytical methods used.

On the other hand, these results differ from those of Das *et al*. (2007) who observed some variation in the moisture, total protein and a considerable variation in the lipid level between un- enriched and enriched *Moina*. The highest lipid content of 20.03% was observed for those enriched with cod liver oil emulsion and the difference between this study and our investigation may be due to the difference in the type of foods and enriched process (Watanabe *et al*., 1982; Leger *et al*., 1987). In this respect, Macedo and Pinto-Coelho (2001) observed that the lipid level of *Moina* varied from 11.4% to 19.9% and this due to feeding effect of different algal diets.

In addition, Mitra *et al*. (2007) recorded lipid content in mixed zooplankton from different ponds varied from 10.79 to 14.55% DM (dry matter) and were inversely related to water temperature. Watanabe *et al*. (1983) analysed various zooplankton; *Daphnia* containing 13% and *Moina* 12-27% lipids whereas in *D. carinata* and *Moina australiensis* it ranged from 7.29-7.73% (Kibria *et al*., 1999).

The amino acid profile of *Daphnia magna* reared in 0‰ (control group) and 0.44‰ synthetic sea water which corresponding to LC10, are shown in Table (6).

It was found that four essential amino acids were detected for *D. magna* reared in 0.44‰ synthetic sea water, while for control group only two essential amino acids (lysine and phenylalanine) were detected. Aspartic acid showed the highest value for both groups and represented by 32.06% for *D. magna* reared in 0.44‰ and 4.43% for control group. On the other hand, aspartic acid, tyrosin and cysteine constituted more than 50% of the total amino acids for group reared in 0.44‰ synthetic sea water. While phenylalanine represented the lowest value (0.974%) for control group and glutamic (0.591%) for those reared in 0.44‰ synthetic sea water. Tryptophan showed approximately similar values for both groups of *D. magna*. Other previous studies revealed that both *D. carinata* and *Moina australiensis* contained appreciable levels of both essential and non-essential amino acids for fish (Kibria *et al*., 1999). However, Yurkowski and Tabachek (1979) and Hepher (1988) reported lower values of some essential amino acids for *Daphnia* and *Moina* sp.

Table (6): Amino acids composition of *Daphnia magna* (% relative concentration

	Amino acids	Control (0‰)	Synthetic sea water (0.44‰)
EAA**	Lysine acid	1.25±0.01	1.84±0.08
	Phenylalanin acid	0.974±0.003	1.785±0.05
	Leucine acid	ND*	0.6850±0.01
	Isoleucine acid	ND*	0.695±0.11
	Tryptophan acid	1.119±0.01	1.241±0.01
Non-EAA***	Tyrosin acid	ND*	6.985±0.52
	Aspartic acid	4.43±0.02	32.06±0.58
	Alanine acid	1.47±0.04	ND*
	Glutamic acid	ND*	0.591±0.06
	Glycine acid	ND*	1.31±0.18
	Cysteine acid	ND*	3.79±0.65
EAA, Non-EAA	Total AA	9.243	50.982
	∑ EAA	2.224	5.005
	∑ Non-EAA	7.019	45.977
	EAA/Non-EAA	0.32	0.11

* Not detected; **Essential amino acids; ***Non-essential amino acids;
Values are given as means ± SE for triplicate determinations

Table (7): Fatty acids composition of *Daphnia magna* (mg/100 gm)

	Fatty acids		Control		Synthetic sea water	
			mg/100 g	%	mg/100 g	%
SFA*	Lauric acid	12:0	1.203±0.12	0.89	1.265±0.03	0.91
	Myristic acid	14:0	2.122±0.01	1.58	2.231±0.02	1.6
	Palmitic acid	16:0	0.465±0.06	0.35	0.489±0.06	0.35
	Stearic acid	18:0	1.225±0.07	0.91	1.288±0.11	0.92
USFA**	Myristolic acid	14:1n-6	1.221±0.13	0.91	1.284±0.06	0.92
	Palmitoleic acid	16:1n-7	8.176±0.58	6.1	8.597±0.19	6.16
	Oleic acid	18:1n-9	4.857±0.47	3.63	5.107±0.58	3.66
	Linoleic acid	18:2n-6	64.457±0.59	48.12	67.778±0.58	48.57
	Linolenic acid	18:۳n-۳	0.488±0.01	0.36	0.514±0.06	0.37
	Arachidonic acid	20:4n-6	47.46±0.58	35.43	48.577±0.09	34.81
	Eicosapentaenoic acid (EPA)	20:5n-3	0.733±0.01	0.55	0.77±0.06	0.55
	Docosahexaeaenoic acid (DHA)	22:6n-3	1.293±0.06	0.97	1.36±0.06	0.97
	Erucic acid	22:1n-9	0.262±0.06	0.2	0.276±0.04	0.2
SFA, USFA	Total FA		133.962		139.536	
	∑ SFA		5.015		5.273	
	∑ UFA		128.947		134.263	
	∑ n-6		113.138	100	117.639	100
	∑ n-3		2.514		2.644	
	∑ PUFA		115.652		120.283	
	n-6/n-3		45.003		44.49	
	EPA/DHA		0.57		0.57	

* Saturated fatty acids; ** Unsaturated fatty acidsValues are given as means ± SE for triplicate determinations

On the other hand, Watanabe *et al*. (1983) reported that *Artemia* nauplii of different origin had different amino acids profiles. Mitra *et al*., (2007) detected all the ten essential amino acids with low level of methionine in mixed zooplankton samples collected from fertilized earthen ponds.

Moreover, fatty acids play a major role as an energy source, affect cellular membrane structure and function, are important for cell growth differentiation and metabolism, improve resistance to stress (starvation and osmotic shock) and regulate gene expression (Kamler *et al*., 2008)

The fatty acids composition of *Daphnia magna* cultured in 0‰ salinity and concentration 0.44‰ of synthetic sea water were shown in Table (7). noticed that myristic acid was the dominant saturated fatty acids followed by stearic acid and lauric acid for control group (0‰S) and those cultured in concentration 0.44‰S, whereas, palmitic acid was found in trace amount. It was found that *D. magna* in both control and those reared in 0.44‰ contain high level of unsaturated fatty acids linoleic acid; omega-6 (48.12% of total fatty acids for control group and 48.57% for those reared in 0.44‰S) and arachidonic acid (20:4n-6) 35.43% for control group and 34.81% for concentration 0.44‰S, this finding in agreement with that obtained by Aman and Altaff (2004) who stated that *Mesocyclop aspericomis* contain high level of these unsaturated fatty acids. Also, the present study is in agreement with Das *et al*. 2007 who reported that the linoleic acid in sunflower oil-enriched *Moina* is much higher than un-enriched *Moina*. It indicates the role of linoleic acid on the growth enhancement of *M. rosenbergii* (Guary *et al*., 1976; kanazawa *et al*., 1977b, 1979c and Read, 1981). Zooplankton contain high levels of arachidonic acid which help in the growth and survival of larvae of turbot fish as documented by Bell *et al*., (1995), Sargent *et al*.(1995) and Tidwell *et al*., (1997). Also in the present study, it was found that *Daphnia* in control and those reared in 0.44‰, not have adequate amount of eicosapentaenoic acid (EPA) (0.55‰ of total FAs) or docosahexaeaenoic acid (DHA) (0.97‰ of total FAs) were found in small amounts. This result was in agreement with that obtained by Lim *et al*. (2000) who found very small amounts of EPA (2.3 mg/g^{-1} dry weight) and DAA ($0.2mg/g^{-1}$ dry weight) in *Artemia* and Das *et al* (2007) with *Moina*. From data presented total fatty acids were similar or slightly different for *D. magna* reared in concentration 0.44‰S (139.536mg/100gm) than those cultured in control (133.962mg/100gm); this similarity may be due to the similar food conditions as reported by Bengtson *et al*. (1991), who stated that the fatty acid composition of *Artemia* nauplii is considered to be more environmentally than genetically determined and reflect the fatty acid profile of the diet direct received by the original adult population (Lavens *et al*., 1989).

On the other hand Das *et al*. (2007) reported that the enrichment of *Moina mircrura* with different oils improve the eicosapentaenoic acid (EPA) and DHA which affect positively on growth and survival of *M. rosenbergii* larvae fed on it. *Daphnia* like *Moina* may be a beneficial alternative to *Artemia* especially in developing countries where imported *Artemia* are costly and sometimes scarce. *Daphnia* spp. is known to be suitable live food source for raising fish and prawn larvae (Masters, 1975; Rasawo and Radull, 1986; Habashy, 1998). However, Abdel Rahman (1996) proved that enrichment of *Artemia* with HPUFA greatly increase growth and survival of fish and shrimp larvae. Fujita *et al*. (1980) clarified that the high mortality observed in red sea bream culture is due to the lack of HUFA in *Artemia* nauplii given as a single food.

Like *Artemia*, *Moina* and *Daphnia* does not meet the requirement of the predator crustaceans with respect to EPA and DHA though it contains 60-70% protein (dry wet). This nutritional quality can be enhanced with HUFA (Das *et al*., 2007).

CONCLUSION

In conclusion, the salinity significantly influenced the growth, survival and reproduction rates of *D. magna* and 0.44‰ of synthetic sea water was the optimal salinity.

Reproduction and survival rates of *D. magna* which lived at the same salinity during study differ with different salinities. The influences of salinity on cumulative progeny per female differed. All rates were greater at LC10 than those at higher salinities. However, daphnids survival rate was best at 0‰ followed by LC10 for the three saline waters studied and decreased gradually with increasing salinities. This study indicated that reproduction of daphnids was favored at the low salinity and survival was favored in control.

The salinity could influence to some extent the time of first reproduction but increased the number of progeny per female at low salinities.

It can be concluded that the best reproduction of *D. magna* occurs at salinity values lower than 3‰.

D. magna like *Moina* can be used as a substitution of *Artemia* in aquaculture. This is because it contains suitable levels of protein, amino acids and unsaturated fatty acids (USFA), and we recommend that its nutritive value can be enhanced by enrichment processes. USFA have been found to be critical for maintaining high growth, survival and reproduction rates and high food conversion

efficiencies for a wide variety of marine and freshwater organisms.

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REFERENCES

1. Abdel Rahman, S.H. (1996). "Nutritional evalution of different *Artemia* nauplii as food for fish and shrimp larvae". Invertebrate zoology and parasitology, V. 19(D), pp. 199–213.
2. Ackman, R. G. (1969). Gas-liquid chromatograph of fatty acids and esters. Methods in Enzymology, San Diego, V. 14, p. 392-381.
3. Aladin, N.V., (1991). Salinity tolerance and morphology of the osmoregulation organs in Cladocera with special reference to Cladocera from the Aral sea. Hydrobiologia 225, 291–299.
4. AOAC (1999). Official method of analysis (16th ed.). Washington (DC): Association of Official Analytical Chemists.
5. Aman, S. and Altaff, K. (2004). Biochemical profile of *Heliodiaptomus viduus*, S*inodiaptomus*(*Rhinediaptomus*) *indicus,* and *Mesocyclops aspericornis* and their dietary evaluation for postlarvae of *Macrobrachium rosenbergii*. Zoological Studies, V. 43, N. 2, pp. 267-275.
6. Arnér, M. and Koivisto, S. (1993). Effects of salinity on metabolism and life history characteristics of *Daphnia magna*. Hydrobiologia 259, 69–77.
7. Baillieut M. and Blust R. (1999). Analysis of the swimming velocity of cadmium stressed *Daphnia magna* – Aquat. Toxicol. 44: 245– 254.
8. Bell, J.G.; Carfell J.D.; Tocher D.R.; Macponald F.M. and Sargent J.R. (1995). Effect of different dietary arachidonic acid: docosahexaenoic acid ratios or phospholoipid, fatty acid composition and prostogland production in juvenile turbot (*Scophthalamus maximum*). Fish. Physiol.Biochem.V.14, pp.139-151.
9. Bengtson, D. A., Léger, P. and Sorgeloos, P. (1991). Use of *Artemia* as a food source for aquaculture. In: Browne, R.A., Sorgeloos, P., Trotman, C.N.A. (Eds), *Artemia* Biology. CRC Press, Boca Roton, Florida, pp. 255-285.
10. Casey, R., Scrimgeour, G. and Kendall,S. (2000). Final report: Effects of water temperature and treated pulp mill effluent on survival and growth of *Daphnia magna* (Cladocera: Daphnidae) and *Taenionema* (Plecoptera Taeniopterygidae) – Alberta Environment Sustainable Forest Management Research Program, Pub no: T/678.
11. Christian, G. (1990). HPLC Tips and Tricks. Great Britain at the Iden Press, Oxford. pp.608.
12. Cowgill, U.M. and Milazzo, D.P. (1990). The sensitivity of two cladocerans to water quality variables, salinity and hardness. Arch. Hydrobiol. 120, 185–196.
13. Cowgill, U.M. and Milazzo, D.P. (1991). Demographic effects of salinity, water hardness and carbonate alkalinity on Daphnia magna and Ceriodaphnia dubia. Arch. Hydrobiol. 122, 35–56.
14. Das, S.k.; Tiwari, V.K.; Venkateshwarlu, G.; Reddy, A.K. and Parhi, J. (2007). "Growth, survival and fatty acid composition of *Macrobrachium rosenbergii* (deMan, 1879) post larvae fed HUFA-enriched *Moina micrura*". Aquaculture, V. 269, pp. 464–475.
15. Daughady, W.H.; Lawry, O.H. and Rosenbrugh, N.J. (1952). Determination of cerebrospinal fluid protein with the Folin phenol reagent. J. Lab. Clin. Med., V. 39, pp. 663-665.
16. Ehlinger G.S. and Tankersley R.A. (2004). Survival and development of horseshoe crab (*Limulus polyphemus*) embryos and larvae in hypersaline conditions – Biol. Bull. 206: 87–94.
17. Fayed, S. E. and Ghazy, M. M. (2000). Toxicity monitoring of water supplies using *Daphnia magna* Straus. Pol. Arch. Hydrobiol. 4(2): 171-188.
18. Finney, D. J. (1977). "Probit Analysis" 3rd Ed., Cambridge University Press, Cambridge, Great Britain, pp. 1-333.
19. Folch, J.; Lees, M. and Sloane-Stanoley, G.H.A. (1957). Simple method for the isolation and purification of total lipids from animal tissue. Journal of Biological Chemistry, Bethesda, V. 226, p. 497-509.
20. Fujita, S., Watanabe, T. and Kitajima, C. (1980). Nutritional quality of *Artemia* from different locations as living feed from the viewpoint of essential fatty acids for marine fish. In: The Brine Shrimp *Artemia* volume3: Ecology, Culturing and Use in Aquaculture (ed. By G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers), pp. 277-290. Universa Press, Stroudsburg, PA.
21. Ghazy, M. M. (1997). Aquatic fauna as a water quality monitoring device. Ph. D.Thesis, Faculty of Science. Cairo University, 169p.
22. Ghazy, M. M. (2003). Water Quality and seasonal succession of phytoplankton and zooplankton in two thermal springs in Cairo, Egypt, J.Egypt. Ger. Soc. Zool. 40A: 169-183.
23. Green, A.J., Fuentes, C., Moreno-Ostos, E. and Rodrigues da Silva, S.L., (2005). Factors

influencing cladoceran abundance and species richness in brackish lakes in Eastern Spain. Ann. Limnol.—Int. J. Limnol. 4, 73–81.
24. Grzesiuk, M. and Mikulski,A. (2006). The effect of salinity on freshwater crustaceans. Pol. J. Ecol. 54(4): 669–674.
25. Guary, J.C., Kayama, M., Murakami, Y.and Ceccaldi, H.J. (1976). The effects of a fat-free diet and compounded diets supplemented with various oils on molt, growth and fatty acid composition of prawn, *Penaeus japonicue* Bate. Aquaculture, V. 7, pp. 245-254.
26. Habashy, M. M. (1998). "Experimental studies on rearing the freshwater shrimp, *Macrobrachium rosenbergii* (De Man) by using certain aquatic insects and zooplankton present in the Nile water at El- Qanater El-Khayriya region". Ph.D. thesis, Faculty of science, Ain Shams university-210pp.
27. Hall, C.J.and Burns, C.W. (2002). Mortality and growth response of *Daphnia carinata* to increases in temperature and salinity – Freshwat. Biol. 47: 451–458.
28. Harder, W. (1968) Reaction of plankton organisms to water stratification – Limnol. Oceanogr. 13: 156–168.
29. Hartman, L. and Lago, R.C.A. (1973). Rapid preparation of fatty acid methyl esters from lipids. Laboratory practice, London, V. 22, p. 475-476.
30. Hebert, P.D.N., Remigio, E.A., Colbourne, J.K., Taylor, D.J. and Wilson, C.C. (2002). Accelerated molecular evolution in halophilic crustaceans. Evolution 56, 909–926.
31. Hepher, B. (1988). "Nutrition of pond fishes. Cambridge Univ. press, Cambridge, NY, USA, 388pp.
32. Kamler, E.; Wolnicki, J.; Kamiński , R. and Sikorska, J. (2008). Fatty acid composition, growth and morphological deformities in juvenile cyprinid, *Scardinius erythrophthalmus* fed formulated diet supplemented with natural food. Aquaculture, V. 278, pp. 69-76.
33. Kanazawa, A., Teshima, S., Kayama, M., and Hirata, M. (1977b). Essential fatty acids in the diet of prawn: I. Effect of linoleic and linolenic acids on growth. Bull. Jpn. Soc. Sci. Fish. V. 43, pp. 1111-1114.
34. Kanazawa, A., Teshima, S., Tokiwa, S., (1979c). Biosynthesis of fatty acids from palmitic acid in the prawns, *Penaeus japonicus*. Mem. Fac. Fish., Kagoshima Univ. V., 28, pp. 17-20.
35. Kefford, B.J., Palmer, C.G., Pakhomova, L., and Nugegoda, D. (2004). Comparing test systems to measure the salinity tolerance of freshwater invertebrates. Water SA 30, 499–506.
36. Kibria, G.; Nugegoda, D.; Fairclough, R.; Lam, P.; and Bradbv, A. (1999). Utilization of wastewater-grown zooplankton: nutritional quality of zooplankton and performance of silver perch *Bidyanus bidyanus* (Mitchell 1838) Teraponidae fed on wastewater grown zooplankton. Aquac. Nutr., V. 5, pp. 221-227.
37. Kikuchi S. (1983) The fine structure of the gill epithelim of a fresh-water flea, *Daphnia magna* (Crustacea: Phyllopoda) and changes associated with acclimation to various salinities – Cell Tissue Res. 292: 253–268.
38. Knight, A.; Anderson, S. and Rowle, J.M. (1972). Chemical basis of the sulfo-phosphovanilien reaction of estimating of totalserum lipids Clin, Chem, 18:3, 199pp.
39. Lagerspetz, K., (1955). Physiological studies on the brackish water tolerance of some species of *Daphnia*, Arch. Soc.Vanamo 9 Suppl: 138-143.
40. Lavens, P., Léger, P. and Sorgeloos, P. (1989). Manipulation of the fatty acid profile in *Artemia* offspring produced in intensive culture system. In: N. De Pauw, E. Jaspers, H. Ackefors and N. Wilkins (eds), Aquaculture Biotechnology in progress. European Aquaculture Society, Bredene. Belgium, pp. 731-739.
41. Leger, P.; Naesens-Foucquaert, E.; Sorgeloos, P.(1987): International study on *Artemia*: xxxv. Techniques to manipulate the fatty acid profile in *Artemia* nauplii, and the effect on its nutritional effectiveness for the marine crustacean *Mysidopsis bahia* (M). In: Sorgeloos, P., Bengston, D.A., Declier, W., Jaspers, E. (Eds.), Artemia Research and its Applications:3. Ecology, Culturing use in Aquaculture. Universa Press, Wetteren, Belgium, pp.441-424.
42. Lim, S.-C., Liou C.-H., Cheng J.-H. (2000) .The role of the antennal glands in ion and body volume regulation of cannulated *Penaeus monodon* reared in various salinity conditions – Comp. Biochem. Physiol. 127A: 121–129.
43. Martínez-Jerónimo F., EÍas-Gutiérrez, M., Suárez-Morales, E. (2004). A redescription of Moina hutchinsoni Brehm, a rare cladoceran (Branchiopoda: Anomopoda) found in remnants of a Mexican saline lake, with notes on its life history. J. Crustacean Biol. 24, 232–245.
44. Martínez-Jerónimo F. and Espinosa-Chávez F.(2005) .Notes on the reproduction and survival of *Moina hutchinsoni* Brehm, 1937 – (Moinidae: Anomopoda) grown in media of varying salinity – Aquat. Ecol. 39: 113–118.
45. Martínez-Jerónimo, F. and Martínez-Jerónimo,L. (2007). Chronic effect of NaCl salinity on a freshwater strain of *Daphnia magna* Straus

(Crustacea: Cladocera): A demographic study, Ecotoxicology and Environmental Safety 67: 411–416.
46. Masters, C.O. (1975). Encylopedia of live foods. T.F.H. Publication, Neptuno City, NJ, 336pp.
47. Mitra, G.; Mukhopadhyay, P.K. and Ayyappan, S. (2007). "Biochemical composition of zooplankton community grown in freshwater earthen ponds: Nutritional implication in nursery rearing of fish larvae and early juveniles". Aquaculture, V. 272, pp. 346–360.
48. Payne M.F. and Rippingale R.J. (2001). Effects of salinity, cold storage and enrichment on the calanoid copepod *Gladioferens imparipes* – Aquaculture, 201: 251–262.
49. Peters, R.H., (1987). Metabolism in *Daphnia*. In: Peters, R.H., de Bernardi, R. (Eds.), *Daphnia*. Mem. Ist. Ital. Idrobiol. 45, 193–243.
50. Potts, W.T.W. and Durning, C.T. (1980). Physiological evolution in the branchiopods. Comp. Biochem. Physiol. 67B:475-484.
51. Rasawo, J. and Radull, J. (1986). Inoculation of brine shrimp, *Artemia salina* in Kenya: Expected impact on aquaculture development. In E.A.Huisman(Ed.), Aquaculture research in *Africa region*. Wageningen, The Nother. lands: Pudoc.
52. Read, C.H.L., (1981). The response of *Penaeus indicus* (Crustacea: Penaeidae) to purified and compound diets of varying fatty acid composition. Aquaculture, V. 24, pp. 245-256.
53. Sargent, J.R.; Bell, J.G.; Bell, M.V.; Handerson, R.J. and Tocher, D.R. (1995). Requirement criteria for essential fatty acids. Journal of Applied Ichthyology, V.11, pp.183-198.
54. SAS Institute, Inc. (1988). SAS User's Guide: Statistics, SAS Institute, Cary, NC, USA.
55. Schuytema, G.S., Nebeker, A.V., and Stutzman, T.W. (1997). Salinity tolerance of *Daphnia magna* and potential use for estuarine sediment toxicity tests. Arch. Environ. Contam. Toxicol. 33, 194–198.
56. Schallenberg M., Hall C.J., and Burns C.W. (2003). Consequences of climate-induced salinity increases on zooplankton abundance and diversity in coastal lakes – Mar. Ecol., Prog. Ser. 251: 181–189.
57. Sprague, J. B. (1969). Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Wat. Res. 3:793-821.
58. Tay, S.H; Rajanshi, V.K.; Ho, W.H; Chew, J.; and Yap, E.A. (1991). Culture of cladoceran *Moina micrura* Kurz using agroindustrial wastes. In: de Silva, S.S.(Ed.), Proceedings of the Fourth Asian Fish Nutrition Workshop, Fish Nutrition Research in Asia, Vijayawada, India. Asian Fisheries Society, Manila, Philippines, pp.135-141.
59. Teschner M. (1995) .Effects of salinity on the life history and fitness of *Daphnia magna* variability within and between populations – Hydrobiologia, 307: 33–41.
60. Tidwell, J.H.; Schulmeister, G. ; Manl, C. and Coyle, S. (1997). "Growth, survival and biochemical composition of freshwater prawn *Macrobrachium rosenbergii* fed natural food organisms under controlled conditions". Journal of the world aquaculture society, V.28, No.2, pp. 123-132.
61. Watanabe, T., Ohta M., Kitajima C. and Fujita, S., (1982). Improvement of dietary value of brine shrimp *Artemia salina* for fish larvae by feeding them on W3 highly unsaturated fatty acid. Bull. Jap.Soc. Sci.Fish. V.48, pp. 1775-1782.
62. Watanabe, T., Kitajima, C. and Fujita, S., (1983). Nutritional values of life organisms used in Japan for mass propagation of fish: a review. Aquaculture, V. 34, pp. 115-143.
63. Williams, W.D. (1998). Salinity as a determinant of the structure of biological communities in salt lakes. Hydrobiologia 381, 191–201.
64. Young G., Bjornsson B.Th., Prunet P., Lin J.R. and Bern H.A. (1989). moltification and seawater adaptation in Coho salmon (Coho: *Oncorhynchus kisutch*): plasma proloctin growth hormone, thyroid hormones, and cortisol – Gen. Comp. Endocrinol. 74: 335–345.
65. Yurkowski, M. and Tabachek, J.L. (1979). Proximate and amino acid composition of some natural fish foods. In: Halver, J.E., Tiews, K.(Eds), proceeding of the world symposium on Finfish Nutrition and fish feed Technology, V. II. Heenemann, Hamburg, pp. 435-448.

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